

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

Claim 1.-109. (Canceled)

Claim 110. (New) A method for diagnosing prostate cancer in a subject suspected of having prostate cancer, comprising:

detecting, in a tissue or body fluid sample from said subject, the presence of an abnormally methylated cytosine in the glutathione S-transferase (GST) Pi gene, wherein the presence of said abnormally methylated cytosine is indicative of prostate cancer; and

wherein said abnormally methylated cytosine is within a CpG site located within a region defined by and including nucleotides 1232 to 1942 (CpG sites +1 to +55) of SEQ ID NO: 60.

Claim 111. (New) The method of claim 110, wherein said abnormally methylated cytosine is within a CpG site located within a region defined by and including nucleotides 1273 to 1942 (CpG sites +9 to +55) of SEQ ID NO: 60.

Claim 112. (New) The method of claim 110, wherein the presence of at least two abnormally methylated cytosines are detected, the presence of which are indicative of prostate cancer.

Claim 113. (New) The method of claim 110, wherein said detecting comprises the steps of:

(i) treating DNA, obtained from a tissue or body fluid sample of said subject, so that unmethylated cytosines in the DNA are converted to uracil or another nucleotide capable of forming a base pair with adenine, while methylated cytosines in the DNA are left unchanged or

are converted to a nucleotide capable of forming a base pair with guanine, wherein said DNA comprises said glutathione S-transferase (GST) Pi gene;

(ii) carrying out an amplification reaction of a target region within said GST-Pi gene using the resulting treated DNA of step (i) as a template, wherein said target region is amplified only when said abnormally methylated cytosine is left unchanged or is converted to a nucleotide capable of forming a base pair with guanine as a result of said treating in step (i), and

(iii) determining if said target region is amplified in step (ii), wherein amplification of the target region is indicative of the presence of said abnormally methylated cytosine in said sample, thereby diagnosing said prostate cancer.

Claim 114. (New) The method of claim 113, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 115. (New) The method of claim 114, wherein said PCR amplification reaction uses a reverse primer having guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with an abnormally methylated cytosine, the presence of which is indicative of prostate cancer, or will form a mismatch with uracil, which is not indicative of prostate cancer.

Claim 116. (New) The method of claim 115, wherein said PCR amplification uses a forward primer having cytosine at at least one site corresponding to an abnormally methylated cytosine, the presence of which is indicative of prostate cancer.

Claim 117. (New) The method of claim 116, wherein the forward and reverse primers are of 12 to 30 nucleotides in length.

Claim 118. (New) The method of claim 117, wherein the forward and reverse primers are selected so as to anneal to a sequence within the target region that includes two to four abnormally methylated cytosines, the presence of which are indicative of prostate cancer.

Claim 119. (New) The method of claim 113, wherein the treatment of DNA obtained from a tissue or body fluid sample of the subject involves reacting the DNA with bisulphite.

Claim 120. (New) The method of claim 116, wherein the amplification involves PCR amplification using primer pairs consisting of a forward and a reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9)

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T, or a mixture thereof; and R is A, G, or a mixture thereof.

Claim 121. (New) The method of claim 116, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and a reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

Claim 122. (New) The method of claim 116, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and a reverse primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T, or a mixture thereof; and R is A, G, or a mixture thereof.

Claim 123. (New) The method of claim 110, wherein said tissue or body fluid sample is blood, blood serum, blood plasma, urine, lymph, or bone marrow.

Claim 124. (New) The method of claim 110, wherein said detecting comprises the steps of:

(i) treating DNA, obtained from a tissue or body fluid sample of said subject, with a restriction endonuclease that recognizes a restriction site within a glutathione S-transferase (GST) Pi gene and which does not cleave at said restriction site when a cytosine in said restriction site is methylated; wherein said abnormally methylated cytosine is within said restriction site; and wherein said DNA comprises a GST-Pi gene;

(ii) carrying out an amplification reaction of a target region of the GST-Pi gene using the resulting treated DNA of step (i) as a template, wherein said target region contains said

restriction site and is amplified only when said restriction site has not been cleaved in step (i) by said restriction endonuclease;

(iii) determining if said target region is amplified in step (ii), wherein amplification of the target region is indicative of abnormal methylation of said cytosine in said sample, to thereby diagnose said prostate cancer.

Claim 125. (New) The method of claim 124, wherein said amplification reaction involves polymerase chain reaction (PCR) amplification.

Claim 126. (New) A method for diagnosing liver cancer in a subject suspected of having liver cancer, comprising:

detecting, in a tissue or body fluid sample from said subject, the presence of an abnormally methylated cytosine in the glutathione S-transferase (GST) Pi gene, wherein the presence of said abnormally methylated cytosine is indicative of liver cancer; and

wherein said abnormally methylated cytosine is within a CpG site located within a region defined by and including nucleotides 1232 to 1942 (CpG sites +1 to +55) of SEQ ID NO: 60.

Claim 127. (New) The method of claim 126, wherein said abnormally methylated cytosine is within a CpG site located within a region defined by and including nucleotides 1273 to 1942 (CpG sites +9 to +55) of SEQ ID NO: 60.

Claim 128. (New) The method of claim 126, wherein the presence of at least two abnormally methylated cytosines are detected, the presence of which are indicative of liver cancer.

Claim 129. (New) The method of claim 126, wherein said detecting comprises the steps of:

(i) treating DNA, obtained from a tissue or body fluid sample of said subject, so that unmethylated cytosines in the DNA are converted to uracil or another nucleotide capable of forming a base pair with adenine, while methylated cytosines in the DNA are left unchanged or are converted to a nucleotide capable of forming a base pair with guanine, wherein said DNA comprises said glutathione S-transferase (GST) Pi gene;

(ii) carrying out an amplification reaction of a target region within said GST-Pi gene using the resulting treated DNA of step (i) as a template, wherein said target region is amplified only when said abnormally methylated cytosine is left unchanged or is converted to a nucleotide capable of forming a base pair with guanine as a result of said treating in step (i), and

(iii) determining if said target region is amplified in step (ii), wherein amplification of the target region is indicative of the presence of said abnormally methylated cytosine in said sample, thereby diagnosing said liver cancer.

Claim 130. (New) The method of claim 129, wherein said amplification reaction involves polymerase chain reaction (PCR) amplification.

Claim 131. (New) The method of claim 130, wherein said PCR amplification reaction uses a reverse primer having guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine, the presence of which is indicative of liver cancer, or will form a mismatch with uracil, which is not indicative of liver cancer.

Claim 132. (New) The method of claim 131, wherein said PCR amplification uses a forward primer having cytosine at at least one site corresponding to an abnormally methylated cytosine, the presence of which is indicative of liver cancer.

Claim 133. (New) The method of claim 132, wherein the forward and reverse primers are of 12 to 30 nucleotides in length.

Claim 134. (New) The method of claim 133, wherein the forward and reverse primers are selected so as to anneal to a sequence within the target region that includes two to four abnormally methylated cytosines, the presence of which are indicative of liver cancer.

Claim 135. (New) The method of claim 129, wherein the treatment of DNA obtained from a tissue or body fluid sample of the subject involves reacting the DNA with bisulphite.

Claim 136. (New) The method of claim 126, wherein said tissue or body fluid sample is blood, blood serum, blood plasma, urine, lymph, or bone marrow.

Claim 137. (New) The method of claim 132, wherein the amplification involves PCR amplification using primer pairs consisting of a forward and a reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCTAAAAACCGCTAACG (SEQ ID NO: 9)

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T, or a mixture thereof; and R is A, G, or a mixture thereof.

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Claim 138. (New) The method of claim 132, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and a reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACGCTAACG (SEQ ID NO: 9).

Claim 139. (New) The method of claim 132, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and a reverse primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T, or a mixture thereof; and R is A, G, or a mixture thereof.

Claim 140. (New) The method of claim 126, wherein said detecting comprises the steps of:

(i) treating DNA, obtained from a tissue or body fluid sample of said subject, with a restriction endonuclease that recognizes a restriction site within a glutathione S-transferase (GST) Pi gene and which does not cleave at said restriction site when a cytosine in said

restriction site is methylated; wherein said abnormally methylated cytosine is within said restriction site; and wherein said DNA comprises a GST-Pi gene;

(ii) carrying out an amplification reaction of a target region of the GST-Pi gene using the resulting treated DNA of step (i) as a template, wherein said target region contains said restriction site and is amplified only when said restriction site has not been cleaved in step (i) by said restriction endonuclease;

(iii) determining if said target region is amplified in step (ii), wherein amplification of the target region is indicative of abnormal methylation of said cytosine in said sample, to thereby diagnose said liver cancer.

Claim 141. (New) The method of claim 140, wherein said amplification reaction involves polymerase chain reaction (PCR) amplification.

Claim 142. (New): A method for diagnosing prostate cancer or liver cancer in a subject, comprising:

detecting, in a tissue or body fluid sample from said subject, the presence of an abnormally methylated cytosine in the glutathione S-transferase (GST) Pi gene, wherein the presence of said abnormally methylated cytosine is indicative of prostate cancer or liver cancer; and

wherein said abnormally methylated cytosine is within a CpG site located within a region defined by and including nucleotides 1232 to 1942 (CpG sites +1 to +55) of SEQ ID NO: 60.